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Fungicidal and Bactericidal Action of Selenium and Tellurium Compounds

BY

NORMAN McKEE STOVER

B.Sc. University of Alberta, 1918
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THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1926

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Fungicidal and Bactericidal Action of Selenium and Tellurium Compounds

IN THE periodic table, the elements selenium and tellurium lie between arsenic and antimony on the one side and the halogens on the other. Since all these bordering elements possess toxic properties, it is not surprising that compounds of selenium and tellurium also show considerable toxicity.

The purpose of this investigation was to determine the possibility of using compounds of selenium or tellurium as bactericides and fungicides to combat such tree diseases as pear blight (*B. amylovorus*), apple blotch (*Benturia inaequalis*), and chestnut blight (*Endothia parasitica*), and also as herbicides against such weeds as dandelions, Canada thistle, burdock, plantain, and pigweed.

The work of such investigators as Joachimoglu,[1] Joachimoglu and Hirose,[2] Lehmann,[3] and Stoklasa[4] has shown that selenium dioxide is more toxic than sulfur dioxide, that selenites and tellurites are more toxic than selenates and tellurates, and that selenites are more toxic than tellurites. If the toxic properties of selenium and tellurium compounds are associated with the ease with which such compounds are reduced, then it is not surprising that selenites are more toxic than tellurites, since selenium compounds are in general more readily reduced than the corresponding tellurium compounds.

The action of selenium and tellurium compounds on bacteria and fungi has been studied by a large number of investigators. The results of many experiments have shown that various compounds of selenium are reduced by bacteria, the reduced selenium being deposited within the cell, coloring it red, while the medium remains colorless. Corresponding tellurium compounds produce a gray deposit when so reduced. Similarly, Turina[5] was able to show that both selenium and tellurium are deposited in the tissues of plants by the reduction of selenites, selenates, tellurites, and tellurates. Several workers have reported that such compounds are

[1] *Biochem. Z.*, **107**, 300 (1920).
[2] *Ibid.*, **125**, 1 (1921).
[3] *Ibid.*, **134**, 390 (1922).
[4] *Compt. rend.*, **174**, 1075, 1256 (1922); *Biochem. Z.*, **130**, 604 (1922).
[5] *Biochem. Z.*, **129**, 507 (1922).

decidedly injurious to the growth of plants. Lougee[6] experimented with selenium compounds with a view to using them to control such tree diseases as apple blotch and pear blight, and also as herbicides against dandelions. The authors have undertaken to continue this work by applying selenium and tellurium compounds to still other weeds and to one other tree disease—namely, chestnut blight.

Preparation of Materials

For the field experiments, approximately 0.1 normal solutions of selenious acid, sodium selenite, and sodium tellurite were made up and served as stock solutions. These were diluted as required to the concentrations to be tried out. Crystals of pure selenious acid were used to make up the solution of selenious acid. Sodium selenite solution was made by dissolving a weighed amount of selenious acid crystals in a small amount of water, making the solution neutral to phenolphthalein by the addition of sodium hydroxide, and then diluting to give an approximately 0.1 normal solution. The sodium tellurite solution was made by dissolving a weighed amount of tellurium dioxide in the least possible amount of sodium hydroxide solution adding hydrochloric acid until a slight turbidity persisted, then just enough alkali again to clear up the turbidity, and finally water to give a 0.1 normal solution.

For the laboratory experiments on chestnut-blight fungus and pear-blight bacteria it was thought desirable to prepare the solutions more carefully and accurately than those used in the field. The following solutions were made and analyzed:

Selenious Acid (H_2SeO_3)—Crystals of the pure acid were used to prepare an exactly 0.1 normal solution, the concentration being checked by an analysis for selenium according to the method of Gooch and Clemons.[7]

Sodium Selenite (Na_2SeO_3)—A weighed amount of twice-sublimed selenium dioxide was dissolved in the volume of sodium hydroxide solution of known concentration required to give sodium selenite. After diluting to the proper volume the solution was found to be 0.0998 normal when analyzed by the method of Lenher and Kao[8].

Potassium Selenite (K_2SeO_3)—A 0 0998 normal solution of potassium selenite was made by the same method as the sodium selenite.

Thallous Selenite (Tl_2SeO_3)—Thallium metal was converted into the normal sulfate (Tl_2SO_4) and the latter dissolved in water and changed into the hydroxide by means of barium hydroxide solution. Phenolphthalein was then added and the solution neutralized with selenious acid. The precipitate of barium sulfate and possibly some barium selenite was filtered off and the filtrate concentrated on the steam bath. The crystals of thallous selenite, which separated out upon cooling, were recrystallized twice from aqueous solution and dried at 105° C. Analyses for both selenium and thallium

[6] *Ind. Eng. Chem.*, **17**, 456 (1925).
[7] *Am. J. Sci.*, **50**, 51 (1895).
[8] *J. Am. Chem. Soc.*, **47**, 2454 (1925).

showed a purity of 99.1 per cent. A 0.1 normal solution of this salt was then made.

LITHIUM SELENITE (Li_2SeO_3)—Pure lithium sulfate was converted into lithium selenite by the same general method used in the preparation of thallous selenite, except that the final product was fused before analysis. Analysis indicated a purity of 99.6 per cent. A 0.1 normal solution was then made up.

SODIUM TELLURITE (Na_2TeO_3) AND POTASSIUM TELLURITE (K_2TeO_3)—Tenth-normal solutions of these salts were made by the same method used in preparing the solution of sodium selenite, already mentioned, but using tellurium dioxide instead of selenium dioxide. The tellurium dioxide was prepared as follows: Tellurium was dissolved in slightly diluted nitric acid and the solution evaporated to dryness. The product was dissolved in sodium hydroxide solution and filtered. The filtrate was acidified with acetic acid, then a faint excess of ammonium hydroxide added, and finally the slightest possible excess of acetic acid again added. The precipitated tellurium dioxide was washed with hot water and dried at 105° C. The product analyzed 99 per cent tellurium dioxide by the method of Lenher and Kao.[8]

TELLURIUM ACID TARTRATE [$Te(HC_4H_4O_6)_4$]—A 5 per cent solution of this compound was made by dissolving the calculated amounts of tellurium dioxide and tartaric acid crystals in the proper amount of water.

Work on Chestnut Blight

Chestnut blight is a tree disease caused by the fungus *Endothia parasitica*. The disease has for some time been seriously threatening the extinction of chestnut trees in the eastern states. Pure cultures of the fungus were obtained from the Bureau of Plant Industry, at Washington, D. C. These were subcultured as growths were needed for the various experiments. All such stock cultures were grown at room temperature on corn-meal agar, the mycelium penetrating the agar and the spore-bearing bodies developing on the surface.

The media used in the various tests on chestnut-blight fungus were prepared according to the following formulas:

CORN-MEAL AGAR

50 grams corn meal
1000 cc. distilled water
Heat on water bath at 58° C. for 1 hour
Filter through filter paper, or centrifuge
Add 1.25 per cent agar (or 13 grams)
Make up to 1000 cc.
Steam for 1.5 hours
Filter, or centrifuge
Tube and autoclave at 115° C. for 16 to 20 minutes

CORN-MEAL WATER EXTRACT

50 grams corn meal
1000 cc. distilled water
Heat on water bath at 58° C. for 1 hour
Filter through filter paper, or centrifuge
Make up to 1000 cc.
Autoclave at 115° C. for 16 to 20 minutes

During the first stages of the investigation it was thought desirable to determine the effects of the various compounds on

mycelium and spores separately. A series of heat tests applied to separate water suspensions of mycelium and spores showed that a temperature of 50° C. for 10 minutes would kill the mycelium but not the spores. This set of conditions was therefore used to obtain suspensions of spores free of living mycelium when it was desired to test the compounds on spores only. However, when the compounds were tried out on spores and mycelium separately, it was found that the spores were less resistant to the chemicals than was the mycelium, probably because the spores would begin to germinate as soon as placed in the solutions, with the result that the compounds would really be acting on very young mycelium rather than on the spores themselves. This is borne out by the fact that during a series of preliminary experiments, using cultures of different ages, the younger growths of the fungus were less resistant to the action of the compounds than old growths. Since it thus seemed impossible to work with spores alone, no further tests were carried out on spores.

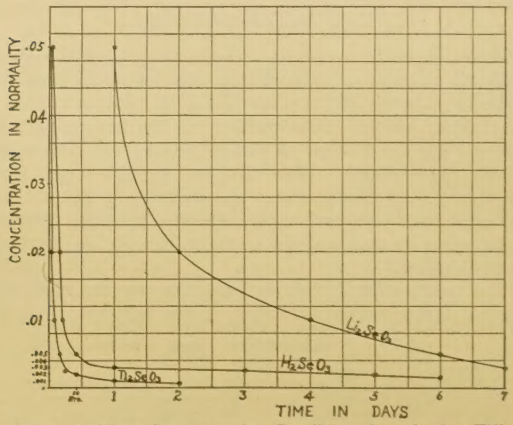

Figure 1—Time-Concentration Conditions Required to Kill Chestnut-Blight Fungus by Selenium Compounds

For the tests on mycelium the fungus was grown in tubes of corn-meal agar. At the end of 14 days the bottoms of about ten tubes of culture were broken off, the exposed agar flamed, and that portion of the medium containing the mycelium cut off with a sterile spatula and transferred to a sterile, glass-stoppered bottle containing sterile glass beads and sterile water. The bottle and contents were shaken until the agar was thoroughly broken up and a uniform suspension obtained. Four cubic centimeters of this suspension were then added to 4 cc. of the various dilutions of each compound to be tried. The solutions actually mixed with the mycelium suspensions were twice

as concentrated as the resulting solutions were desired. Four cubic centimeters of suspension added to 4 cc. of sterile distilled water served as a control tube in each series. All tubes were shaken to mix the contents and duplicate transfers made from each tube into sterile, corn-meal water extract at the ends of definite time periods. Two 4-mm. loopfuls were used for each transfer. The tubes were read one week after the last 7-day transfer was made. Each compound was tried out over a range of dilutions varying from 0.05 to 0.0005 normal.

The results of the tests showed that sodium selenite, sodium tellurite, potassium selenite, potassium tellurite, and tellurium acid tartrate had practically no fungicidal effect, for the concentrations used, over a period of 7 days. Selenious acid, lithium selenite, and thallous selenite were, however, quite toxic. The relative toxicities of the three compounds are shown by the curves in Figure 1, representing the conditions of time and concentration required to kill the fungus. The thallium compound was made and tried because thallium compounds are known to be toxic and thallous selenite happens to be a soluble salt (selenites of the heavy metals are insoluble).

Since it was thought that these compounds might later be applied by the method of tree injection (Lipman[9] and Rumbold[10]), it remained to be determined whether the compounds would go into circulation in the sap of the chestnut trees. Accordingly, the cut ends of chestnut twigs were dipped into selenious acid solutions of concentrations 0.02, 0.002, and 0.001 normal for several days. Control twigs were supported in tap water only. From three to five leaves were picked from each twig, dried, and analyzed for the presence of selenium by the method of Schmidt,[11] as follows:

> The dried leaves were powdered and digested with 10 cc. of concentrated nitric acid until only 2 or 3 cc. remained. Then another 10 cc. of nitric acid were added and the digestion and evaporation to 2 or 3 cc. repeated. About 5 cc. of concentrated sulfuric acid were next added and the mixture boiled, a few drops of nitric acid being added from time to time to hasten the oxidation. When a straw-colored solution was finally obtained, the boiling was continued until dense, white fumes were evolved. The solution was then cooled, enough concentrated sulfuric acid added to bring the volume up to 10 cc., and 0.01 gram of codeine added. The presence of selenium was indicated by the formation of a green color, changing gradually to a blue-green and finally to a pure blue color.

Such analyses showed the presence of selenium in all the leaves from the twigs which had stood in the selenious acid solutions, but no selenium was found in the control leaves.

[9] *Science*, **60**, No. 1561, p. x (1924); *Calif. Countryman*, **12**, No. 2, 9 (1925).

[10] *Phytopathology*, **5**, 225 (1915); *Am. J. Botany*, **7**, 1, 44 (1920).

[11] *Arch. Pharm.*, **252**, 161 (1914).

Work on Apple Blotch

During the spring and summer of 1925 some work was done on apple trees infected with apple blotch (*Benturia inaequalis*). Small areas on the previous year's twigs infected with the disease were painted with 0.1 and 0.04 normal solutions of selenious acid. Even after eight treatments the results were very conflicting. Some areas ceased increasing in size while others continued to spread. Of the control areas, not treated, some continued to spread while others stopped. Hence, no definite results were obtained by treating apple blotch in the field by this method. No laboratory tests were tried on the fungus as in the case of chestnut-blight fungus.

Work on Pear Blight

Laboratory experiments were also conducted to determine the toxicity of various selenium and tellurium compounds toward the bacteria causing pear blight (*B. amylovorus*). Pure cultures of this organism were obtained from Prof. H. W. Anderson, of the Department of Pomological Pathology of the University of Illinois. These were subcultured as needed. The medium used for culturing the pear-blight bacteria was a beef-extract broth containing 1 per cent dextrose, and was prepared by the following formula:

BEEF-EXTRACT BROTH

3 grams beef extract
5 grams peptone
1000 cc. distilled water
Boil until ingredients are dissolved
Adjust solution to neutrality by adding NaOH or dilute HCl, using brom-thymol blue as indicator
Filter through filter paper until clear
Add 10 grams dextrose (C. P.)
Tube and sterilize

Cultures 48 hours old, grown at room temperature in the beef-broth medium, were used for the tests. Ten cubic centimeters of such a culture were mixed with 60 cc. of sterile broth, and 4 cc. of this suspension of bacteria were added to 4 cc. of each dilution of the solutions to be tested as in the case of the tests on chestnut-blight fungus. At the ends of definite time periods duplicate transfers were made from each tube into fresh, sterile broth, one 4 mm. loopful being used for each transfer. The tubes were read 3 days after the last 7-day transfer was made. The compounds used were selenious acid and thallous selenite, each being tried out in concentrations varying from 0.05 to 0.0005 normal. The relative toxicities of the two compounds are shown by the curves in Figure 2, the curves representing the conditions of time and concentration required to kill the bacteria.

In order to ascertain whether selenium solutions would enter the circulatory system of the pear tree, experiments

were carried out on pear twigs as already described in the work on chestnut blight. The leaves of twigs which had stood in selenious acid solutions of various concentrations showed the presence of selenium, when analyzed, but none was found in control leaves which had stood in water only.

It was next decided to determine the effect of solutions of selenious acid on pear trees themselves, using the method of tree injection similar to that used by Lipman.[1] Young, healthy pear trees were selected for the experiments. A 0.5-inch hole was bored about half way through the trunk of a tree. Into this hole was inserted a rubber stopper carrying a glass tube about 3 inches in length. The glass tube was pushed through the stopper so that the end of the tube came flush with the end of the stopper. The projecting end of the glass tube was connected by a piece of rubber tubing to a 5-liter aspirator bottle supported 4 or 5 feet above the point at which the injection was to be made into the tree. The bottle was filled with the solution to be used and while the solution was flowing from the end of the glass tube carrying the rubber stopper the latter was inserted firmly into the hole in the tree. With this procedure no air became trapped in the hole when the solution entered. The first tree injected took in about 3 liters of 0.005 normal selenious acid during one afternoon. In the course of a day or two all the leaves on those branches directly above the point of injection turned black and eventually became dry and crisp. The leaves on the opposite side of the tree were hardly affected, if at all, showing that very little lateral diffusion occurred as the solution entered the circulatory system. A few weeks later the tree put forth new leaves on those branches which had been affected by the solution. It was clear from this experiment, however, that a concentration of 0.005 normal was injurious to the tree. An analysis of the blackened leaves showed the presence of selenium. A second tree was injected with about 3 liters of 0.001 normal selenious acid. This time the injury to the leaves was much less intense, although still too severe to permit the use of such a solution for controlling pear blight.

The ends of a few branches of a third tree were inoculated with pear-blight bacteria, but only a few of the inoculations spread, probably owing to the unfavorable weather conditions prevailing at the time. One branch which showed the characteristic spreading of the disease was injected with about 500 cc. of 0.001 normal selenious acid, but it was observed later that the disease had not been checked, and a very slight injury had been done to the leaves on that particular branch. Owing to the unfavorable weather conditions no further experiments were carried out along this line.

Experiments similar to those described under apple blotch were also tried on pear trees showing the presence of pear

blight. Here, again, the results obtained were so indefinite that no conclusions could be drawn.

Work on Weeds

Preliminary work on dandelions showed that solutions of sodium selenite and sodium tellurite were hardly injurious even in 0.1 normal concentration, while selenious acid seemed to be much more toxic. Lougee[6] had previously found that 0.05 to 0.025 normal solutions of selenious acid killed dandelions after two sprayings, two days apart, but did not affect grass or clover, while 0.02 normal selenious acid caused only the leaves of dandelions to shrivel. Preliminary experiments conducted by the present authors indicated that 0.05 and 0.02 normal solutions of selenious acid were very injurious to grass and although dandelion leaves were completely shriveled, new shoots soon developed from the old roots. It therefore seemed desirable to carry out further experiments with selenious acid with a view to determining how dilute a solution could be used and still be effective, and also to see if a concentration could be found which would kill dandelions without injuring grass.

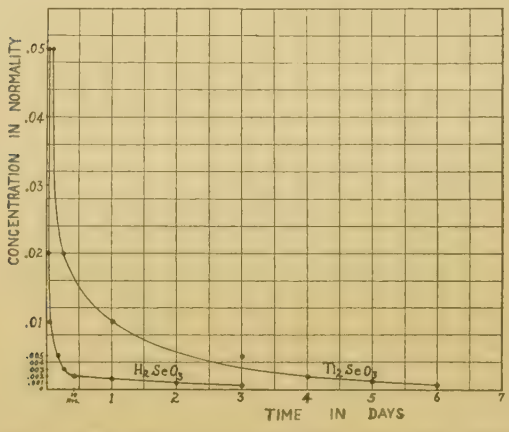

Figure 2—Time-Concentration Conditions Required to Kill Pear-Blight Bacteria by Selenium Compounds

During the summer of 1925 large healthy dandelion plants were sprayed with solutions of selenious acid ranging in concentration from 0.05 to 0.0025 normal. Ten plants were sprayed with each solution, ten treatments being given over a period of 49 days. Sprayings were made at 4-day intervals. A second set of plants was sprayed at 2-day intervals. Still a third set was treated, but this time sprayings were made at night to see if sunlight played any part in the toxicity of the selenious acid. Stated briefly, the

results showed that 0.05 normal selenious acid did not permanently injure the dandelions after ten sprayings. The highest dilution which was successful in killing the original leaves and preventing new leaves from developing to normal size was 0.005 normal. No difference could be observed in the effects produced by spraying in the daytime or at night, or in the results obtained by spraying at 2-day or at 4-day intervals.

It is not impossible that weather conditions may play a very important part in such experiments. Pammel[12] mentions the importance of such weather conditions as cloudiness and relative humidity. The summer of 1925 was unusually dry, which may explain why the present authors obtained results somewhat different from those obtained by Lougee.

In view of the results given above, it would seem probable that, under normal weather conditions, selenious acid in a concentration of 0.005 normal could be used as a spray to check the growth of dandelions in lawns and yet not permanently injure grass.

The results of similar work on other weeds may be summed up as follows: Canada thistle was killed by spraying in the late fall with 0.02 normal selenious acid, whereas summer spraying did not prove successful. Burdock was readily killed by 0.02 normal selenious acid by spraying in midsummer. For plantain and pigweed, selenious acid in concentrations of at least 0.05 normal were required to actually kill the plants. The results of experiments on poison ivy were not definite.

Summary

The fungus causing chestnut blight (*Endothia parasitica*) was exposed to the action of solutions of sodium selenite, sodium tellurite, potassium selenite, potassium tellurite, and tellurium acid tartrate in concentrations varying from 0.05 to 0.0005 normal for a period of 7 days. Practically no fungicidal action was observed. Selenious acid, lithium selenite, and thallous selenite, however, were quite toxic.

Tenth and 0.04 normal solutions of selenious acid were not effective in controlling apple blotch (*Benturia inaequalis*) when the infected areas on twigs were repeatedly painted with the solutions.

Laboratory tests show selenious acid and thallous selenite to be quite bactericidal toward pear blight (*B. amylovorus*). Field tests conducted on pear trees to control this disease have thus far been unsuccessful.

Experiments have shown that solutions of selenious acid will enter the circulatory system of pear and chestnut trees, so that selenium solutions may be used for tree injection

[12] "Weeds of the Farm and Garden," p. 102 (1911).

experiments if desired. A successful method of tree injection is described in detail.

Selenious acid is sufficiently toxic to be considered as a possible herbicide against such weeds as dandelions, Canada thistle, and burdock.

VITA

The author was born September 3, 1895, at Norwich, Ontario. His early education was received in Ontario and Saskatchewan. In 1918 he graduated from the University of Alberta with the degree of B.Sc., and in 1921 received the degree of M.Sc. from the same institution. From 1918 to 1923 the writer held the position of full-time assistant in Chemistry at the University of Alberta, and since 1923 has been a part-time assistant in Chemistry at the University of Illinois. He is a member of Phi Lambda Upsilon and Sigma Xi.